AWARD NUMBER: W81XWH-12-1-0287

TITLE: Improving the Diagnostic Specificity of CT for Early Detection of Lung Cancer: 4D CT-Based Pulmonary Nodule Elastometry

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14. ABSTRACT

In this study we propose to develop and validate pulmonary nodule elastometry imaging, a method complementary to CT that has the potential to increase the specificity of screening for early detection of lung cancer. We propose to address the need for the greater specificity in lung cancer screening by characterizing a mechanical property of pulmonary lesions, specifically pulmonary nodule (PN) elasticity, in addition to standard anatomic features. We hypothesize that malignant and benign PN can be distinguished more specifically by different elasticities determined from 4D CT images. The specific aims of the study were the development of pulmonary nodule elastometry algorithms based on deformable image processing of 4D CT images and their validation in an animal model and in a retrospective review of over 200 4D CT scans from patients with small malignant pulmonary nodules previously treated with radiation in our department. We have successfully developed algorithms, and in a first validation we have demonstrated proof of principles that elastometry can distinguish malignant PNs from surrounding lung tissue (a manuscript is in preparation). The validation in animal models and the retrospective analysis of the human data is ongoing.

15. SUBJECT TERMS
lung cancer, radiotherapy, biological effectiveness, high energy electrons

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Contract number: W81XWH-12-1-0287

Title: Improving the Diagnostic Specificity of CT for Early Detection of Lung Cancer: 4D CT-Based Pulmonary Nodule Elastometry

Principal Investigator: Billy W Loo Jr, MD PhD

Introduction:

In this project we are addressing a shortcoming of existing lung cancer screening methods by developing a CT based method of characterizing a mechanical property of pulmonary lesions, specifically tissue elasticity (stiffness) that should have a higher specificity than purely anatomic low-dose CT. It is the aim of the proposed study to decrease the false positive rate of CT screening by analyzing the mechanical properties of suspiciously appearing tissue during CT screening. We hypothesize that malignant pulmonary nodules are less elastic (stiffer) than benign nodules and that this difference in elasticity can be used to differentiate cancerous from benign nodules, which would help to decrease the false positive rates of CT screening. A measure of elasticity can be derived from high-resolution 4-dimensional computed tomography (4D CT) using deformable image registration algorithms. Unlike conventional 3D CT imaging that results in a static image of the scanned anatomy, 4D CT incorporates also the temporal changes of the anatomy caused by respiratory motion, yielding a CT 'movie' that allows the evaluation of tumor motion and the calculation of the elasticity.

Body:

Specific Aim 1. Development of deformable image algorithms for processing the 4D CT images to determine the elasticity of malignant and benign pulmonary nodules. *(**Dr. Maxim**, Tasks 1, months 1 – 8)*

Task 1. Development of the software for deformable image registration, analysis of the DVF and the calculation of the elasticity parameter (Matlab).

*The software will be developed using the mathematical package Matlab (The Mathworks Inc., Natick, MA). Two deformable image registration algorithms will be used (DIR^{vol} and a method based on optical flow, DIR^{OF}). The resulting displacement vector fields will be analyzed and an elasticity parameter for the pulmonary nodules will be calculated (**Dr. Maxim**, months 1 – 8).*

Status (Task 1):

A manuscript describing our algorithm and its validation was accepted for publication in 'Radiotherapy and Oncology' and is attached to this report.

Specific Aim 2: Validate our method in rat models of human lung cancer and benign inflammatory lesions. *(**Dr. Maxim**, Tasks 2-4, months 3 – 24)*

Task 2. Preliminary experiments: Establish optimal protocol for the benign pulmonary model (granulomatous inflammation) and study growth kinetics.

*2a. Purchase animals: Rowett rats, A549 and SK-MES-1 cells from American Tissue Culture Collection (ATCC), carbon nanotubes (catalogue number 900–1501, lot GS1801), SES research (Houston, TX) and necessary culturing media. (**Dr. Maxim**, months 1-3)*

*2b. Inoculate 15rats (Rowett nude rats) with carbon nanotubes and follow with serial MicroCT measurements to study growth kinetics to establish the time for nodule development to reach desired size. (**Dr. Maxim**, 15 rats total, months 3 – 6)*

Task 3. Grow orthotopic model of lung cancer and benign lesions and follow with serial MicroCT imaging: preliminary experiments to establish protocol and optimize software

*3a. Inoculate 10 rats with orthotopic human lung cancer cells (A549, left lung) and carbon nanotubes (right lung) (**Dr. Maxim**, months 7-9)*

*3b. Acquire CT images at peak-inhale and peak-exhale using a small animal ventilator (**Dr. Maxim**, month 9-10)*

*3c. Analyze CT images and derive elasticity parameter and optimize software if necessary. (**Dr. Maxim**, month 10)*

Task 4. Grow orthotopic model of lung cancer and benign lesions and follow with serial MicroCT imaging, analyze data

*4a. Inoculate remaining 40 rats (A549 cells, left lung in Rowett nude rats) and follow with CT imaging at peak-inhale and peak-exhale (**Dr. Maxim**, months11-13)*

*4b. Perform simplified analysis: Delineate malignant and benign pulmonary nodules and measure volumes at peak-inhale and peak-exhale. Derive elasticity parameter based on the ratio of the volumes. (**Dr. Maxim**, months 14-15)*

*4c. Analyze acquired CT images and derive elasticity parameter by analyzing the displacement vector fields and perform statistical analysis. (**Dr. Maxim**, months 16-18)*

*4d. Repeat experiments and analysis with second cancer cell line (SK-MES-1), 50 Rowett rats, (**Dr. Maxim**, months 18-23)*

*4e. Publish animal study results (**Dr. Maxim**, month 24)*

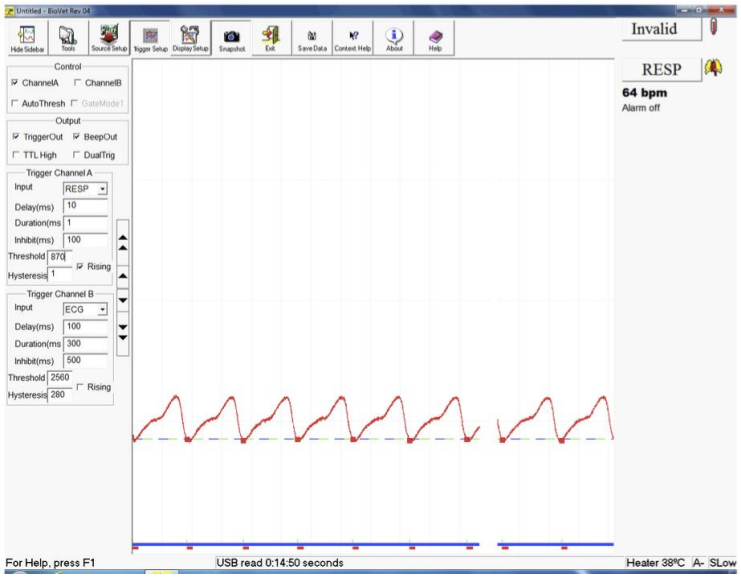
Status (Tasks 2, 3, 4): Status (Tasks 2, 3, 4): Prolonged repairs on the GE-MicroCT scanner have delayed our proposed experiments, however the defects were fixed and we were able to acquire the proposed 4DCT images and perform the analysis.

Figure 1: Position of mouse with pressure belt in CT scanner.

Figure 1 shows a mouse placed in the CT scanner with the pressure sensing belt around its chest that provided the respiratory signal to the scanner.

Figure 2 shows a typical respiratory trace and and a sagittal CT image overlay of acquisitions acquired at maximum inhale and exhale, respectively. Pulmonary nodules (benign and malignant) were delineated by Dr. Loo and analyzed as described in the manuscript attached to this report (Negahadar 2014).

We were able to to successfully generate two benign pulmonary nodule models using talc and matrigel. The calculated elasticities for tumors, talc and matrigel are



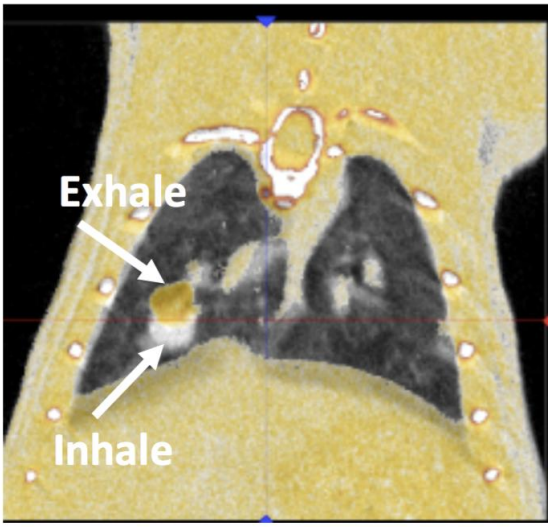


Figure 2: Typical respiratory trace used for acquisition of 4DCT images (left). Right, sagittal 4DCT images acquired at maximum inhale and exhale. Arrows point to nodule location within the lung at exptrme tidal breathing volumes.

shown in Figure 3, demonstrating that our proposed method can distinguish between tumors (formed by A549 lung cancer cells) and matrigel. There was no statistically difference in elasticity between tumors and talc.

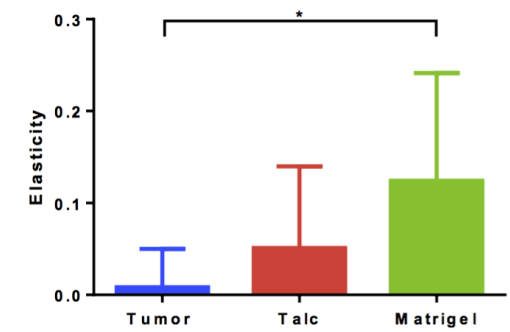


Figure 3: Calculated elasticities of talc, matrigel (benign model)) vs. tumors (A549 lung cancer cells). * indicates statistical difference at $p<0.05$. Error bars indicate SD, N=11.

To validate our results, we performed electronic force microscopy (AFM) on excised samples to verify the elasticities determined by 4DCT image analysis. Figure 4 shows the acquisition technique and derived Young modulus (a metric for tissue elasticity) for tumors,

matrigel and lungs. Similar to the elasticity derived from 4DCT image analysis, the Young modulus for tumors was higher for tumors compared to matrigel and lungs, validating the results derived from 4DCT image analysis. Figure 5 summarizes the results of the AFM derived Young modulus.

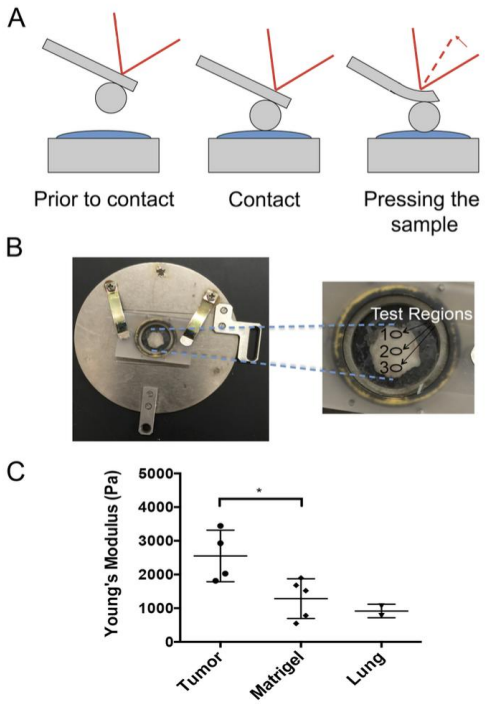


Figure 4: Experimental setup and measurements of the Young modulus via AFM (A and B). Determined Young modulus vis AFM for tumors, matrigel based nodules and lung shows statistical difference between tumors and matrigel, demonstrating the successful ability of 4DCT image based analysis to distinguish malignant vs. benign pulmonary nodules. Errors bars indicate SD, N=4

In summary, we have demonstrated that pulmonary nodule elasticities derived from 4DCT images are able to distinguish benign from malingnants nodules. The results were verified by mechanical measurments of the Young modulus which correlated well with the elasticities derived by 4DCT.

A manuscript summarizng our methods and resultsand aknowledging DoD support, is in preparation and will be submitted to 'Radiotherapy and Oncology' and made available to the DoD as soon as it is accepted.

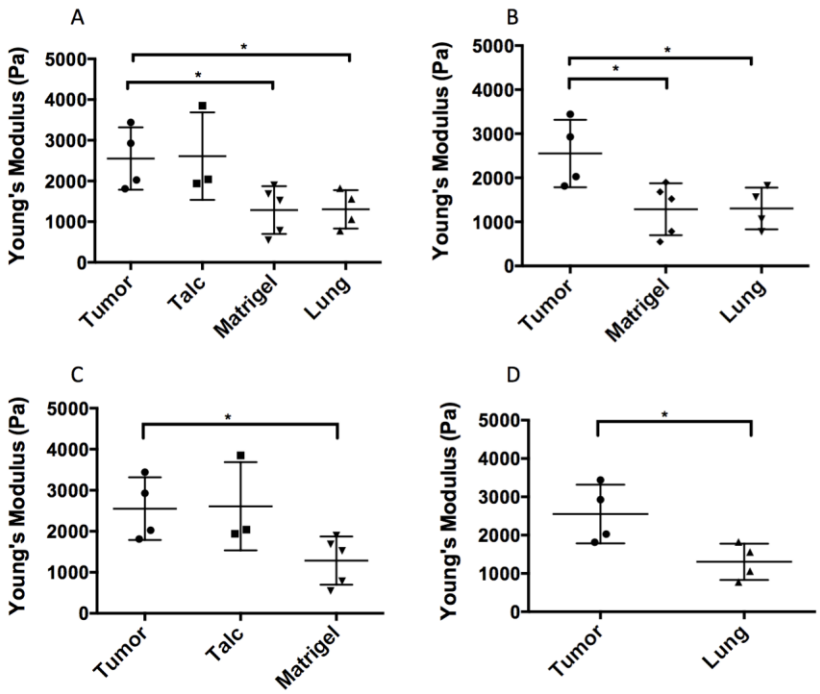


Figure 5: Derived Young modulus via AFM for matrigel, talc and lung tumor models. Statistiacal difference denoted by * is indicated at the $p<0.05$ level. N=4 per group.

Specific Aim 3: Validate our method in a retrospective review of over 200 4D CT scans from patients previously treated in our department. *(**Dr. Loo**, Task 5 months 1 – 20)*

Task 5. Analyze approximately 200 4D CT images from previously treated patients and patients recruited within the funding period.

*5a. De-archive all previously acquired thoracic 4D CT scans and identify suitable patients for the study. Our institutional data (all 4D CT scans) are currently stored on DVD's. Data will be de-archived and suitable lung cancer patients (patients with benign and malignant pulmonary nodules) will be identified. (**Dr. Loo**, months 1 – 3)*

*5b. Identify benign and malignant pulmonary nodules to be included in the analysis and delineate nodules at each respiratory phase. (**Dr. Loo**, month 4)*

*5c. Perform simplified analysis by calculating the ratio of the volumes with respect to peak-inhale. (**Dr. Loo**, months 5-8)*

*5d. Analyze all 4D CT images and derive elasticity parameter by analyzing the displacement vector fields and perform statistical analysis (**Dr. Loo**, months 9-15)*

*5e. Analyze data from new patients acquired during the award period (**Dr. Loo**, months 15-18).*

*5f. Publish human study results (**Dr. Loo**, months 19-20)*

Status (Task 5): Due to difficulties identifying benign pulmonary nodules that were suitable for the proposed elastometry analysis, we have analyzed the correlation between malignant pulmonary elastometry and aggressiveness of the tumors based on clinical outcomes, which is an alternative way of determining the discriminatory potential of elastometry and clinically relevant in its own right.

From September 2005 to October 2012, patients who received stereotactic ablative radiotherapy (SABR) for early stage non-small cell lung cancer were included in this study. All patients had a 4D-CT completed at the time of simulation. The extreme inhale and exhale phases were used to calculate the tumor volume ratios (TVR), ring volume ratios (RVR) and elasticity of the primary tumors based on deformable image registration as described in our manuscript (Negahadar 2014). Statistical analyses were performed to identify the clinical parameters and tumor characteristics that correlated with patient outcomes.

In total, 83 patients with 93 lesions met criteria for analysis. Median age at diagnosis was 76 (range: 42-99) and the male/female ratio was 36/46. The majority of lesions were peripherally located (68%) and of adenocarcinoma histology (57%). Median total dose was 50Gy (range: 25-66) in 4 fractions (range: 1-5). Median follow-up was 38 months (range: 5-108) and the local, regional and distant control rates were 84%, 71%, and 70%, respectively.

The median elasticity was 3.43 (range: 0.03-100). Decreased elasticity was a significant independent predictor of regional recurrence either in isolation or usually in combination with distant and/or local recurrence (p=0.036) for both univariate and multivariate analyses. There was no correlation of elasticity with isolated local recurrence, loco-regional recurrence, distant recurrence, or progression free survival. The TVR was a significant predictor of progression free survival (p=0.05). There was no statistically significant correlation of any of the analyzed clinical parameters with overall survival.

Elasticity of early stage non-small cell lung cancer is a significant independent predictor of recurrence involving or mediated by regional nodal metastasis. This knowledge is useful to predict the aggressiveness of early stage lung cancer and may play a role in the selection of treatment options.

A manuscript acknowledging DoD support is in preparation and will be submitted to a peer reviewed journal shortly.

Key Research Accomplishments:

Our first aim was to develop and validate an automated software package for determining PN elasticity against a manual contouring method, and preliminarily assess its ability to distinguish malignant tissue by comparing the elasticities of malignant PN with those of the lung. This work is now completed and a manuscript detailing the methodology and the results was accepted in 'Radiotherapy and Onclogy' (and attached to this report). In animal models we have demonstrated proof of principle that 4DCT derived pulmonary elasticity is able to distinguish malignant from benign nodule as hypothesized in this proposal. Our clinical data has demonstrated proof of principle that elasticity of early stage lung cancer is a significant independent factor for regional recurrence.

Reportable Outcomes:

The following abstracts have been selected for presentation at ASTRO and AAPM:

1. Mohammadreza Negahdar, Billy W Loo, Maximilian Diehn, Lu Tian, Dominik Fleischmann, and Peter G Maxim, *"Automated Tool for Determining Pulmonary Nodule Elasticity to Distinguish Malignant Nodules,"* ASTRO 2014

2. Mohammadreza Negahdar, Billy W Loo, Maximilian Diehn, Lu Tian, Dominik Fleischmann, and Peter G Maxim, "*Comparison of Four Dimensional Computed Tomography (4D CT) versus Breath Hold Images to Determine Pulmonary Nodule Elasticity*", AAPM 2015

The abstracts are included in the 'Supporting Documentation' section.

Conclusion:

We have successfully accomplished the specific aims of the proposed study. We now have functional software to process and analyze 4DCT images to distinguish malignant and benign PN. We have demonstrated proof of principles that pulmonary nodule elasticities derived from 4DCT images are able to distinguish malignant vs. benign nodules, that could be used as a screening tool for early stages of lung cancer patients. Our results were verified and validated by mechanical measurements of the Young modulus via AFM. Our clinical data has demonstrated proof of principle that PN elasticity can predict for regional recurrence. Given these results, we have successfully completed our proposed project.

Supporting Data:

Abstract submitted to the Annual Conference of ASTRO (2014):

Automated Tool for Determining Pulmonary Nodule Elasticity to Distinguish Malignant Nodules

Purpose: To develop and validate an automated method of determining pulmonary nodule (PN) elasticity against a manual contouring method, and preliminarily assess its ability to distinguish malignant tissue by comparing the elasticities of malignant PNs treated with stereotactic ablative radiotherapy (SABR) with those of the lung.

Methods: We analyzed breath-hold images of 30 patients with malignant PNs who underwent SABR in our department. A parametric nonrigid transformation model based on multi-level B-spline guided by Sum of Squared Differences similarity metric was applied on breath-hold images to determine the deformation map. The Jacobian of the calculated deformation map, which is directly related to the volume changes between the two respiratory phases, was calculated. Next, elasticity parameter will be derived by calculating the ratio of the Jacobian of the PN to the Jacobian of a 1cm region of lung tissue surrounding the tumor (E-ROI) as well as the Jacobian of the whole lung (E-Lung).

Results: For the first group of 15 patients we evaluated the volumetric changes of PNs and the lung from the maximum exhale phase to the maximum inhale phase, whereas the reverse was done for the second group of 15 patients. For the first group, mean and standard deviation for E-ROI and E-Lung were 0.91±0.09 and 0.86±0.18, respectively, which was verified by the manual method. For the second group, E-ROI and E-Lung were 1.34±0.27 and 1.57±0.51, respectively. These results demonstrate that the elasticity of the PNs was less than that of the surrounding lung ($p<0.0037$).

Conclusion: We developed an automated tool to determine the elasticity of PNs based on deformable image registration of breath-hold images. The tool was validated against manual contouring. Preliminarily, PN elastometry distinguishes proven malignant PNs from normal tissue of lung, suggesting its potential utility as a non-invasive diagnostic tool to differentiate malignant from benign PN.

Abstract submitted to the Annual Conference of AAPM (2015):

Comparison of Four Dimensional Computed Tomography (4D CT) versus Breath Hold Images to Determine Pulmonary Nodule Elasticity

Purpose: Elasticity may distinguish malignant from benign pulmonary nodules. To compare determining of malignant pulmonary nodule (MPN) elasticity from four dimensional computed tomography (4D CT) images versus inhale/exhale breath-hold CT images.

Methods: We analyzed phase 00 and 50 of 4D CT and deep inhale and natural exhale of breath-hold CT images of 30 MPN treated with stereotactic ablative radiotherapy (SABR). The radius of the smallest MPN was 0.3 cm while the biggest one was 2.1 cm. An intensity based deformable image registration (DIR) workflow was applied to the 4D CT and breath-hold images to determine the volumes of the MPNs and a 1 cm ring of surrounding lung tissue (ring) in each state. Next, an elasticity parameter was derived by calculating the ratio of the volume changes of MPN (exhale:inhale or phase50:phase00) to that of a 1 cm ring of lung tissue surrounding the MPN. The proposed formulation of elasticity enables us to compare volume changes of two different MPN in two different locations of lung.

Results: The calculated volume ratio of MPNs from 4D CT (phase50:phase00) and breath-hold images (exhale:inhale) was 1.00±0.23 and 0.95±0.11, respectively. It shows the stiffness of MPN and comparably bigger volume changes of MPN in breath-hold images because of the deeper degree of inhalation. The calculated elasticity of MPNs from 4D CT and breath-hold images was 1.12±0.22 and 1.23±0.26, respectively. For five patients who have had two MPN in their lung, calculated elasticity of tumor A and tumor B follows same trend in both 4D CT and breath-hold images.

Conclusion: We showed that 4D CT and breath-hold images are comparable in the ability to calculate the elasticity of MPN.

Accepted manuscript in Radiotherapy and Oncology:

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

Form Approved
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Expires Jan 31, 2008

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE ABOVE ORGANIZATION. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT *(X one)*
Billy Loo Jr. MD PhD	W81XWH-12-1-0287	Jamie Brazell	W81XWH-12-1-0287	a. INTERIM [ ] b. FINAL [X]
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THE LELAND STANFORD JUNIOR UNIVERSITY 450 Serra Mall Stanford, CA 94305-2004	20120705	820 Chandler Street Fort Detrick, MD 21702-5014		a. FROM 20120715 b. TO 20150614

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) *(Last, First, Middle Initial)* a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS *(X)* d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER *(X)* e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
None	None	None						

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR *(Last, First, Middle Initial)* N/A	(2) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*	(1) TITLE OF INVENTION	(2) FOREIGN COUNTRIES OF PATENT APPLICATION
(b) NAME OF EMPLOYER N/A	(b) NAME OF EMPLOYER		
(c) ADDRESS OF EMPLOYER *(Include ZIP Code)* N/A	(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS *(Include ZIP Code)* b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES *(YYYYMMDD)* f.	
			(1) CLAUSE NUMBER	(2) DATE *(YYYYMM)*		(1) AWARD	(2) ESTIMATED COMPLETION
N/A							

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate))* [ ] **SMALL BUSINESS** or [ ] **NONPROFIT ORGANIZATION**

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL *(Last, First, Middle Initial)*	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Billy Loo Jr. MD PhD	Associate Professor, Radiation-Oncology		20151228